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Assessment of BoviPureTM for the In Vitro Production of Bovine Embryos

Abstract
The objective of this study was to examine the potential utility of a commercially available sperm separation and purification product for the in vitro production of bovine embryos. Bovine oocytes were purchased from a commercial supplier, and matured oocytes were randomly allocated to one of two treatments. Oocytes were co-incubated with frozen-thawed semen washed twice with BoviPureTM (BoviPure group) or with modified Brackett-Oliphant medium (control group). After a 6-hour insemination period, oocytes were cultured in vitro for 8 days. Cleavage rate of embryos was determined 48 hours post-insemination, and blastocyst formation rate was assessed on day 8 of culture. The experiment was replicated three times, and data were analyzed using chi-square analysis. Washing of sperm in BoviPureTM had no effect (P>.05) on either cleavage rate (77.2%) or blastocyst development (21.6%) when compared with controls (71.9% and 17.1%, respectively). These results indicate that, under conditions of our study, the washing of sperm with BoviPureTM did not significantly enhance the ability to produce bovine embryos in vitro.

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Assessment of BoviPure™ for the In Vitro Production of Bovine Embryos

A.S. Leaflet R1768

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Summary

The objective of this study was to examine the potential utility of a commercially available sperm separation and purification product for the in vitro production of bovine embryos. Bovine oocytes were purchased from a commercial supplier, and matured oocytes were randomly allocated to one of two treatments. Oocytes were co-incubated with frozen-thawed semen washed twice with BoviPure™ (BoviPure group) or with modified Brackett-Oliphant medium (control group). After a 6-hour insemination period, oocytes were cultured in vitro for 8 days. Cleavage rate of embryos was determined 48 hours post-insemination, and blastocyst formation rate was assessed on day 8 of culture. The experiment was replicated three times, and data were analyzed using chi-square analysis. Washing of sperm in BoviPure™ had no effect (P>.05) on either cleavage rate (77.2%) or blastocyst development (21.6%) when compared with controls (71.9% and 17.1%, respectively). These results indicate that, under conditions of our study, the washing of sperm with BoviPure™ did not significantly enhance the ability to produce bovine embryos in vitro.

Introduction

The ovaries of every cow contain tens of thousands of oocytes (eggs), yet most females produce only a handful of offspring during their lifetime--a number far below their biological potential. By harvesting oocytes from the ovarian follicles and maturing and fertilizing them in vitro, it is theoretically possible to produce hundreds of bovine embryos from a single animal.

Since the birth of the first calf produced via in vitro fertilization in 1981, thousands of calves have been born following the transfer of *in vitro*-produced embryos to recipient females. Oocytes may be obtained from living donors using ultrasound-guided oocyte retrieval methods, or they may be obtained by aspiration from ovaries collected at the time of a female's death or slaughter. After in vitro maturation, oocytes can be fertilized with sperm obtained from a genetically elite bull. Fertilized eggs can then be cultured in vitro for approximately one week until the embryo develops enough so that it may be transferred to a recipient female. These techniques allow genetically elite animals to produce live offspring, even in instances where they may not be capable of reproducing under normal mating conditions.

Whenever embryos are produced in vitro (i.e., in a petri dish), the viability of the resultant embryos is often compromised because conditions aren't ideal. Ways to improve aspects of in vitro embryo production are needed. One important aspect of the in vitro embryo production process is sperm preparation. After the freezing and thawing of bovine semen, many sperm are non-viable and/or deformed. In order to maximize chances for fertilization, oocytes should be co-incubated only with viable, normal sperm. Recently, a sperm separation and purification product formulated specifically for use with bull sperm became available; however, the potential usefulness of this product for in vitro production of bovine embryos has not been fully investigated. The objective of this study was to evaluate the use of BoviPure™ for the in vitro production of bovine embryos.

Materials and Methods

Bovine oocytes were purchased from a commericial supplier (TransOva Genetics, Sioux Center, IA). Each week, approximately 200 oocytes were placed into oocyte maturation medium and were shipped in a temperature-controlled environment. On arrival at the laboratory, oocytes were removed from the shipping unit and were placed inside an incubator at 39°C in an atmosphere of 5% CO_2 in air.

BoviPure™ colloid concentrate and BoviPure™ buffer (both from Nidacon International AB, Gothenburg, Sweden) were used to prepare eight solutions, each of a different density ranging from 1.05 to 1.12 g/mL. After preparation, the solutions were warmed to 35°C (in order to avoid any potential cold-shocking of the sperm) before layering (0.85 ml per layer) in a 15 ml centrifuge tube. The solution containing the highest percentage of BoviPure™ colloid concentrate was placed on the bottom, and the remaining solutions were sequentially added in order of progressively decreasing density.

Three straws of frozen-thawed semen from a single Angus bull were centrifuged in a 15 ml centrifuge tube at 500 g for 20 minutes at 35°C to concentrate the sperm. The density gradient previously described was overlaid with 0.7 ml of concentrated sperm solution (containing $45x10^6$ spermatozoa) and was centrifuged at 250 g for 30 minutes at 30°C. After centrifugation, fluid above the sperm pellet was removed before 10 ml of BoviPure™ wash were added. The sperm were mixed before the tube was centrifuged at 600 g for 10 minutes at 35°C. After centrifugation, fluid above the sperm pellet was removed, and 7 ml of semen preparation medium (modified Brackett-Oliphant medium

containing heparin) were added. Sperm were resuspended in this medium before the tube was centrifuged at 1800 rpm for 7 minutes at 30°C. After centrifugation, all fluid in excess of 1 ml was removed. The sperm pellet was resuspended in that 1 ml of solution, sperm were counted using a hemocytometer, and sperm were diluted to a final concentration of $5x10^6$ sperm per ml. Fifty µl of diluted sperm were added to each 50 µl drop of fertilization medium (modified Brackett-Oliphant medium supplemented with 1% BSA) containing oocytes that had been washed previously in fertilization medium.

For the control group, a single straw of semen from the Angus bull used previously was thawed and placed in 7 ml of semen preparation medium. The sperm were centrifuged for 7 minutes at 1800 rpm at 30°C. Fluid above the sperm pellet was aspirated, and 7 ml of semen preparation medium containing heparin were added. Sperm were resuspended and were centrifuged again at 1800 rpm for 7 minutes at 30°C. Fluid in excess of 1 ml total volume was removed. Sperm were resuspended and were counted using a hemocytometer before dilution to a concentration of $5x10^6$ sperm per ml. As before, 50 µl of diluted sperm were added to each 50 µl drop of fertilization medium containing oocytes that had been washed previously in fertilization medium.

Oocytes and sperm were co-incubated for 6 hours. Presumptive zygotes were freed of cumulus cells by vortexing for 5-6 minutes, washed 3 times, and placed in CR1aa culture medium. Plates were incubated at 39°C in a gaseous atmosphere of 5% CO_2, 5% O_2, and 90% N_2. Two hundred fifty µl (half of the total volume) of CR1aa culture medium were removed from each well, and 250 µl of fresh CR1aa culture medium were added at 48 hours following fertilization. One hundred twenty hours following fertilization, 250 µl of CR1aa culture medium were removed from each well and were replaced with 250 µl of CR1aa culture medium containing 5.56mM glucose and 10% fetal bovine serum.

After 48 hours in culture, cleavage rate of embryos was assessed. On day 8 of culture, blastocyst development was assessed. The experiment was replicated three times, and data were analyzed by chi-square analysis.

Results and Discussion

No difference (P>.05) in cleavage rate or blastocyst formation rate was observed between treatments. Of the 324 oocytes co-incubated with sperm washed with BoviPure™, 250 (77.2%) were fertilized and 54 of 250 cleaved zygotes (21.6%) developed to the blastocyst stage. Of the 260 oocytes that were co-incubated with sperm washed with modified Brackett-Oliphant medium, 187 (71.9%) were fertilized and 32 of 187 cleaved zygotes (17.1%) developed to the blastocyst stage.

The in vitro production of bovine embryos involves four basic steps—in vitro oocyte maturation, in vitro sperm preparation, in vitro fertilization of matured oocytes, and in vitro culture of fertilized eggs to more advanced stages of preimplantation embryonic development. None of these procedures is 100% efficient, and failure at one step precludes success at subsequent steps in the collective process.

Our study focused on the second step of the in vitro embryo production procedure—sperm preparation. There are two major aspects of the sperm preparation procedure. One aspect is the in vitro capacitation of sperm, a procedure involving the removal of "protective" proteins from the surface of the sperm that ordinarily prevent the sperm from prematurely fertilizing an oocyte. In the live animal, sperm capacitation ordinarily occurs in the female reproductive tract near the junction of the uterine horn with the oviduct. In vitro, we accomplished the removal of these proteins by treating the bull sperm with heparin. The second aspect of the sperm preparation procedure is the segregation of morphologically normal, motile sperm from abnormal, non-motile sperm. We accomplished this task in vitro by using the commercially available bull sperm separation and purification product called BoviPure™.

BoviPure™ is a proprietary concentrated density gradient for the separation and purification of sperm from bovine semen. To use a density gradient for sperm separation and purification, solutions such as sucrose, Percoll®, or BoviPure™ are prepared at various concentrations and layered (one on top of another). Sperm are placed on top of the layers, and the sperm are "forced" through the different solutions (each of a different density) via centrifugation. In this experiment, we prepared several different layers in an attempt to physically separate morphologically normal, motile sperm from other sperm. Sperm separated/washed in this manner were then used for in vitro fertilization. Cleavage rate and blastocyst formation rate were not statistically different between treatments and controls.

The approach we used in this study (8 layers) differed from the manufacturer's recommended use of BoviPure™ (2 layers). We purposefully did so in an attempt to get maximal separation of sperm with varying physical properties. Our deviation from recommended protocol may have influenced the results because the height/volume of the layers and the force of centrifugation interact to determine the nature and extent of sperm cell preparation. Thus, these results should be interpreted with some caution. Further research will undoubtedly provide greater insight into the desirability of multiple density gradients for separation and purification of bovine sperm. These results indicate that, under the conditions of our study, washing of sperm with BoviPure™ did not significantly enhance in vitro production of bovine embryos.

Implications

If the in vitro production of bovine embryos from genetically elite females is to become more widespread, better techniques must be developed that will lead to improved blastocyst formation rates. In this study, we investigated one potential avenue of accomplishing that, namely alternative methods for sperm preparation and fertilization. The approach used in this experiment, however, did not enhance in vitro embryo production rates. Further research is needed on alternative methods to improve the in vitro production of bovine embryos.